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On the Peculiar Morphology of a Trypanosome from a Case of Sleeping Sickness and the Possibility of its being a New Species. (T. rhodesiense.)

BY J. W. W. STEPHENS, M.D. (CANTAB.), D.P.H., AND H. B. FANTHAM, D.Sc. (LOND.), B.A. (CANTAB.).

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On the Peculiar Morphology of a Trypanosome from a Case of Sleeping Sickness and the Possibility of its being a New Species (T. rhodesiense).

By J. W. W. STEPHENS, M.D. Cantab., D.P.H., and H. B. FANTHAM, D.Sc. Lond., B.A. Cantab.

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(Communicated by Prof. Major R. Ross, F.R.S. Received July 19, 1910.)

[PLATE 6.]

Prefatory Note.

As already stated in a report to the Advisory Committee for the Tropical Diseases Research Fund, dated May 9, 1910, I noticed early in February, 1910, while examining in class work a stained specimen of rat's blood infected with what was supposed to be *T. gambiense*, a marked peculiarity in the morphology. This peculiarity was so striking that I doubted whether the trypanosome with which I was dealing was really *T. gambiense*. On making enquiries I was told that the strain was derived from a case of Sleeping Sickness then in Prof. Ross's clinic in the Royal Southern Hospital, Liverpool. To make certain that there was no error in this statement I myself infected a rat from the patient's blood. The same forms were, however, again encountered. After convincing myself that these forms were constantly present in infected rats, and that they were not shown by the rats infected with the old laboratory strain of *T. gambiense* maintained at the Runcorn Laboratory, I decided through pressure of work to ask Dr. Fantham (now working in the Liverpool School of Tropical Medicine, under funds allotted by the Advisory Committee for the Tropical Diseases Research Fund) to be so good as to assist me in the description of the morphology of this trypanosome. The following paper is the outcome of our joint work.—[J. W. W. Stephens.]

History of the Strain.

The trypanosomes used during this investigation were obtained from W. A., male, aged 26, a native of Northumberland, who was infected in North-East Rhodesia in September, 1909. It is necessary to set forth the itinerary of W. A. while in Africa, as he was never actually in an area infested with *Glossina palpalis*, so far as records are available, and indeed was never nearer (Kasama) than some 86 miles from such an area.

He first went to South Africa in July, 1904, living in Johannesburg till the end of 1906. He then went to Salisbury for two years. About the end of November, 1908, he left Salisbury for North-Eastern Rhodesia, with a view

to prospecting for minerals. The party consisted of two Europeans and ten natives. On the journey northwards he passed through Fort Jameson, Landazi, and Chinsali to Kasama, where he arrived about the beginning of June, 1909. During this northward journey, W. A. passed through an area infested by *Glossina morsitans.* He stayed two months at Kasama, a place from which we have no records of any species of *Glossina.** On the return journey he passed through Mpika (where *Glossina morsitans* occurs), Serenje (no records of *Glossina*), and Mzaza (where there is *Glossina morsitans*). He left Mzaza on September 10, and travelling along the Luangwa River he reached Feira on September 28. During this part of the journey he would pass through an area infested by *Glossina fusca*, between Mzaza and Hargreaves.

He first fell ill on September 20, but after a rest of two days continued his journey. A short stay was made at Feira and then the return journey was continued through the Hartley district to Salisbury, where it was found that he and one native were suffering from trypanosomiasis—parasites being found in his blood in Africa on November 17, 1909.

The patient stated that he had never been near either Lake Tanganyika, Lake Mweru or the Luapula River, which are known to be infested by *Glossina palpalis.* He thought, himself, that he contracted trypanosomiasis while travelling along the Luangwa River, between Mzaza and Feira. The Luangwa Valley is heavily infested with *Glossina morsitans,* but *Glossina palpalis* has not yet been found along the course of this river.

Dr. Bagshawe's article, already noted, on "The Transmission in Nature of *Trypanosoma gambiense,*" should be consulted as to cases of Sleeping Sickness contracted in areas infested by *Glossina morsitans.*

We may note that the case of W. A. has been studied by R. Ross and D. Thomson,† who have found a regular periodical increase in the numbers of the trypanosomes in the peripheral blood of the patient from day to day.

It is also of interest to record that the Rhodesian strain of trypanosome from W. A. is somewhat more virulent to rats and guinea-pigs than the old laboratory strain of *T. gambiense,* a fact already confirmed by other workers in the laboratory. Further, this Rhodesian trypanosome is resistant to atoxyl.

Morphological Features.

It may be stated at once that the peculiarity of this Rhodesian trypanosome is that among the stout or stumpy forms some have the *nucleus at the posterior* (*non-flagellar*) *end.*

* Bagshawe, S.S. Bulletin, No. 18, June, 1910, p. 197.

† 'Roy. Soc. Proc.,' B, 1910, vol. 82, pp. 411–415.

As will be seen from the accompanying figures (Plate 6, figs. 2 to 10), the position of this "posterior" nucleus varies. Starting from the stumpy forms in which the nucleus is in the middle (fig. 1), we have all transitions (figs. 2 to 8) up to that in which the nucleus is actually terminal (fig. 9) and posterior to the blepharoplast (kinetonucleus).

We may meet here any objections that may be raised that the trypanosomes with posterior nucleus are due to distortion, dried films being used, because—

1. We have never found them, though persistently looked for, in films from the same animals infected with the old laboratory strain of *T. gambiense* (figs. 21 to 25), treated in the same way, *i.e.* dried films;

2. Further, we have examined the trypanosomes under discussion, by *intra vitam* staining with methylene blue—by this method the posterior position of the nucleus can be seen; and finally,

3. We have fixed wet films with sublimate-alcohol and with osmic vapour respectively, and subsequently stained them with hæmatoxylin, and found the same forms.

Rats inoculated with the Rhodesian strain usually show a few long thin trypanosomes in the peripheral blood in about three days. The stumpy forms of trypanosomes with the nucleus posterior (figs. 2 to 10) appear about the fifth or sixth day, and from this time onwards somewhat increase in number up to the seventh to eleventh day. They then form about 6 per cent. of the trypanosomes present, but may decrease again, varying from day to day.

These stumpy forms with posterior nucleus (as depicted in figs. 2 to 8) are 17μ to 21μ long and 2μ to 3μ broad. The nucleus often shows a karyosome, and when at the level of the blepharoplast is often kidney-shaped (fig. 8.). There is a well marked blepharoplast (kinetonucleus) and a definite undulating membrane with a flagellar border which terminates in a very short free flagellum. The cytoplasm of these forms is granular, especially at the anterior (flagellar) end, where coarse granules are seen in life, which granules are found to be chromatoid in nature on staining (figs. 2, 3, 5, and 8). These cytoplasmic characters are very like those seen in ordinary stumpy forms of *T. gambiense* (fig. 25). Sometimes a stained line is clearly seen in the stout forms, joining the blepharoplast to the nucleus (fig. 10). We have little evidence as to the relationship of these forms with posterior nucleus to the stumpy forms with the nucleus in the ordinary (central) position (fig. 1), so content ourselves with pointing out that the existence of trypanosomes with posterior nuclei is quite characteristic of this Rhodesian strain, and is so marked a feature that we are always able in films to distinguish it from the old laboratory strain of *T. gambiense*.

These forms with the posterior nuclei are not confined to rats (30 "passages"), but occur also in *Macacus rhesus* (observed by Dr. Yorke) rabbits, and guinea-pigs infected with this strain. We have been unable to detect these forms in the case of Sleeping Sickness itself, for the number of trypanosomes present in the blood was always extremely scanty, and in infected rats showing an equally small number of trypanosomes we have also never been able to find them.

A subsidiary but perhaps not unimportant point in the Rhodesian strain is that many of the ordinary long forms have an elongated posterior end, and may be termed "snout" forms (figs. 13 to 17). The "snout" forms occur more especially during the first half or two-thirds of the period of infection in rats. These elongate forms, while not absent in the old laboratory strain of *T. gambiense*, yet are certainly more numerous in the Rhodesian strain, and even by means of this feature we are able to distinguish the strains. "Snout" forms were frequently seen in the blood of W. A. (figs. 14, 16), and in guinea-pigs. Bruce and Nabarro* have already noted these forms with elongate posterior ends.

Again, although forms with the blepharoplast terminal (figs. 18, 19) are found in the Rhodesian strain, they are less numerous than similar trypanosomes with terminal blepharoplast in the old laboratory strain.

We may also note in passing that pear-shaped (fig. 11) or rounded forms with little or no free flagellum are not uncommon in the Rhodesian strain.

Summary and Conclusions.

1. We attach some importance to the fact that the patient (now dead) from whom this strain was derived was never, as far as careful enquiries could elicit, in a *Glossina palpalis* area, but had been in many *Glossina morsitans* areas, and very probably in a small *Glossina fusca* area.

2. These posterior nuclear forms (figs. 2 to 10) have not, as far as we are aware, been described either in the blood of Sleeping Sickness patients or in the blood of animals infected with *T. gambiense*. Seeing how many competent observers have worked with *T. gambiense*, this is a striking point and can hardly be an oversight.

3. We ourselves have been unable to find posterior nuclear forms, though constantly searched for, in the blood of rats infected with the old laboratory strain of *T. gambiense*.

4. Posterior nuclear forms exist also, as is well known, in *T. transvaaliense*, and by Lühe these are regarded as developmental stages of *T. theileri*.

* Royal Society : Reports of the Sleeping Sickness Commission, No. I, Plate 2, fig. 4.

Whether this be so or not, the condition is not parallel to the one under discussion, for the differences in *T. theileri* are characteristic of all strains of this trypanosome, but the forms which we have described in this paper have, we believe, never been seen before in any strain of *T. gambiense* in man or animals.

5. We are aware that parasitic flagellates may exhibit morphological variations due to changes in metabolism or to differences in environment. However, this Rhodesian strain of trypanosome with posterior nucleus was seen in rats infected from the patient before he was treated with drugs. At first sight also it might appear that the posterior nucleus denoted merely approaching division or even assumption of the round form. However, we have seen division of these posterior nuclear forms (fig. 12). In any case the peculiar morphological feature exhibited by the Rhodesian trypanosome, in the possession of a posterior nucleus, has not been recorded before.

As to the meaning to be attached to these forms, we will consider the following main possibilities :—

(*a*) That we are dealing with a "variety" or "local race" of *T. gambiense* due to some change of environment. The possibility in this case exists of *T. gambiense* having been conveyed by a species of *Glossina* other than *Glossina palpalis*, which may account for the peculiar morphology we record ; but it must be added that we have no knowledge of any other trypanosome undergoing similar changes owing to such a cause. That it is a "laboratory" variation we think is out of the question, as all the known laboratory strains of *T. gambiense* are practically identical in morphology.

(*b*) That we are dealing with a new species of trypanosome also producing Sleeping Sickness in man. In support of this view there is firstly, the morphology, which considered alone is strong evidence, since the Rhodesian trypanosome differs from *T. gambiense* more than, for instance, *T. brucei* does from *T. evansi*. Secondly, there is the history, taken in connection with the fact that, so far as we know, *T. gambiense* is conveyed solely by *Glossina palpalis*, but even should it prove that *Glossina palpalis* exists in the districts through which W. A. travelled, then all the more probable is it that we are dealing with a new species of trypanosome.

It seems to us, however, that it is difficult in the absence of further knowledge to discriminate with certainty between these views. Yet, on account of its peculiar morphological features, this Rhodesian trypanosome at least merits a distinct designation as *T. gambiense rhodesiense*, adopting the trinomial nomenclature. Our own view, however, is that we are dealing with a new species of human trypanosome for which we propose the name *Trypanosoma rhodesiense*.

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EXPLANATION OF PLATE No.

All figures drawn with Abbé camera lucida, using 2 mm. apochromatic objective and 12 compensating ocular (Zeiss). Magnification 1800 diameters approximately.

Figs. 1–20.—Rhodesian strain of human trypanosome. Figures drawn from parasites in the blood of rats, except where otherwise stated.

Fig. 1.—Stout form with nucleus median.

Figs. 2–8.—Stout and stumpy forms, each with posterior nucleus. The nucleus is seen gradually to become more posterior, till it lies behind the blepharoplast (fig. 9).

Fig. 10.—Posterior nuclear form with line connecting blepharoplast and nucleus.

Fig. 11.—Posterior nuclear form becoming rounded.

Fig. 12.—Posterior nuclear form showing division.

Figs. 13–17.—"Snout" forms. (Figs. 13, 15 from rat's blood, figs. 14, 16, 17 from man.)

Figs. 18, 19.—Forms with terminal blepharoplast.

Fig. 20.—Multiple division form, with four blepharoplasts and two nuclei. Such parasites are not uncommon in the Rhodesian strain.

Figs. 21–25.—Various trypanosomes drawn from the blood of rats infected with the old laboratory strain of *T. gambiense*.

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